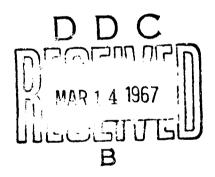PRESENT PROBLEMS IN THE EXPERIMENTAL STUDY OF TYPHUS FEVER AND THE FUTURE
PATH OF ITS DEVELOPMENT

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PRESENT PROBLEMS IN THE EXPERIMENTAL STUDY OF TYPHUS FEVER AND THE FUTURE
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A unique group of transmissive infections, known under the name of
"rickettsioses", have come under study comparatively recently. All told a-
round 70 years have passed since the moment when the Russian surgeon Mochut-
kovskiy (1876), infected himself with the blood of a typhus patient, and
demonstrated the presence of an infectious agent. A little more than 30
years have passed since da Rocha-Lima (1916) established the etiological
significance of the microorganisms, detected in the intestines of lice in-
fected with typhus. Over the years, as a result of the intensive work of
a whole group of investigators, the problem of typhus developed into a
new problem -- the problem of rickettsioses (Monteyro, 1932). Along with
epidemic typhus, which remained an indelible track in the history of humanity,
a new group of related infections was discovered and studied. This group
received the nam of "endemic typhuses" on the basis of its strong bond with
the sites of circulation of rodents -- reservoirs of the virus, or of
anthropods -- the carriers.

However, as usual the leading place in the group of rickettsioses is
occupied by typhus, the carrier of which is the body louse -- which in turn
is intimately connected with man biologically.

Stemming from the leading role of typhus, we are undertaking the task
of sheding light on new data and contemplating the path of future investiga-
tions primarily in the area of this infection.

Following the works of da Rocha-Lima (1916), Topfer (1916), Epshteyn
(1920) and others, many investigators continued for a number of years to have
doubts of the etiological role of rickettsioses. At the present time this
unique group of microorganisms has been assigned a prominent place in the
infectious pathology of man. Combining itself with the properties of
true bacteria and the so-called filterable viruses, the group of rickettsias,
comprising more than 50 representatives, still remains puzzling in many res-
pects for microbiologists. The position of rickettsias in the system of
microorganisms has not been fully understood in the works of recent years, in
which very fine biochemical methods and the electron microscope have been used.

1.

Morphological investigations (Plotz, Smadel, Anderson, Chambers, 1943; Weiss, 1943; Eyer and Ruska, 1944; Pleshkov, 1944) have shown that the structure of rickettsias is in many ways similar with the structure of the bacterial cell.

By electron microscope examination of rickettsias, the presence of a membrane was established (boundary membrane) as well as the presence of specific granule-nucleoids. The possibility is not excluded that the granules are analogous to the diffuse nuclear substance of bacteria. Indirect confirmation of this is the detection in rickettsia of thymo- and ribonucleic acids (Tovarnitskiy and Krontovskaya et al., 1946).

Further investigations in the area of morphology led to still another discovery which connected rickettsias with bacteria. In a series of rickettsia strains, which were cultivated in the intestines of body lice, Pshenichonov, Kazakova and Mitrofanova established the presence of a clearly expressed capsule. This discovery, which was made with the help of contrast (negative) staining of the preparations with fuchsin, was confirmed almost simultaneously in the works of American authors (Mudd, Shepard, 1946) who used an electron microscope. Shepard (1946) not only confirmed the presence of a capsule, but also established that the capsular substance is destroyed following treatment with ether at room temperature. At the same time he demonstrated that during centrifuging the disrupted substance remains in the liquid phase and, due to a distinct antigenic activity, enters into the reaction of the deflection of complement in the presence of immune typhus sera. Apparently this capsular substance is a "soluble antigen" and was detected by Topping and Shear (1945) when treating rickettsias with ether by the method of Craigie.

The "soluble antigen" behaves like pseudoglobulin. It is soluble in distilled water, physiological solution, 8% cold ethyl alcohol and 20% ammonium sulfate. On the other hand, 25% ethyl alcohol and 40--45% ammonium sulfate causes its precipitation. During the immunization of animals, the soluble antigen causes the formation of complement fixing antibodies, enters into the neutralization reaction with immune sera, thus creating an immunity both to the virus and (for rickettsia Mooser) to the toxic substance. It is possible that it also possesses the properties of allergen, since according to the data of Mayevskiy (1945) the lysate of rickettsia produces a positive cutaneous test in typhus patients. Finally, apparently this same capsular substance is the thermolabile antigen of rickettsia. The latter, according to data from Topping and Shear (1944) and others, is disrupted by heating the rickettsia up to $60°$, and is the carrier of type specific and immune properties, while the thermostable antigen is the same in rickettsias of epidemic and murine typhus, that is, devoid of type specificity. The sera of guinea pigs and rabbits, immunized with the thermostable antigen (heated rickettsias) do not contain neutralizing antibodies, while immunized guinea pigs remain susceptible to a virulent strain of rickettsia. In the opinion of Felix (1943) the thermostable antigen of rickettsia conforms to the O-antigen of Proteus X_{19}, and the thermolabile antigen is probably analogous to

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the Vi-antigen of the typhus bacillus. As Felix points out, the thermolabile antigen is toxic, and possesses the capability of causing the formation of opsonins in the organism, and prevents an interaction between the O-antigen and O-antibodies. In this manner the thermolabile antigen protects the rickettsia from the bactericidal effect of natural O-antibodies and those which have been created as a result of immunization. Apparently in connection with the "soluble" and "thermolabile" antigens is the toxic substance of rickettsia, which was detected by Gildemeister and Haagen (1940). These authors accidentally discovered that following administration to white mice, Rickettsia mooseri, cultivated in the vitelline sac of chick embryos causes the death of the mice over a period of several hours prior to the development of the infection. They assumed the presence of a specific toxin in the cultures of rickettsias. Subsequently Otto and Bickhardt (1942), Bengston, Topping and Henderson (1942), Mayevskiy, (1945), Shishkina and Raykher (1945), Bronshteyn (1945), Groupe and Donovick (1945), Hamilton (1945), Fitzpatrick (1946) and others established that the so-called toxic substance may be detected in rickettsia by any method of cultivation -- in vitelline sacs, pulmonary tissue, and the intestines of lice. The toxin substance is precipitated during centrifugation together with the bodies of rickettsias, but does not pass through a rickettsia retaining filter, and is destroyed by heating up to 60° for 30 minutes. It loses toxicity rapidly in the presence of formalin, ether and carbolic acid and at pH=8 and higher (Shishkina and Raykher, 1945). The toxic substance possesses definite antigenic properties and is easily neutralized with immune sera (Mayevskiy, 1944; Bronshteyn, 1945).

It is interesting to note that according to Groupe and Donovick (1945), the toxic substance is not identical to the complement-fixing substance which is described by Topping and Shear under the name of "soluble antigen", since the latter is not destroyed under the action of formalin and ether. By observing rickettsias developing in the intestines of typhus lice, Shishkina and Raykher (1945) showed that the toxic substance is produced in the lice parallel to the multiplication of rickettsias and disappears parallel with their death. These same facts were also noted by Groupe and Donovick (1924) for rickettsias developing in a vitelline sac. In the old reports by Weigl (1924), there are indications that R. prowazeki is toxic for lice. Actually the test of feeding lice on the epidermomembrane with blood containing massive doses of rickettsias (50--100--200 intestines of typhus lice per 1 cm^3 of blood) showed that the lice died in 12-24 hours with the no apparent destruction of the cells of the intestine, while the control lice, which received the same amount of intestines of healthy lice, remained alive. The observations by Kligler and Olejnik (1944) are the only different case. They apparently were able to obtain toxic substances which did not contain rickettsias by means of freezing and thawing or by centrifugation. The last circumstance presents doubts in the reliability of their data, since in contrast to the old opinion of Rocha-Lima (1916) and Weigl (1924), the investigations of recent years showed that fine "pulverized" forms of rickettsias observed during the various methods of cultivating are capable of passing through a Berkefeld filter (Raykher and Kruger, 1942; Muromtsev, 1944; Bengston, 1945).

3.

There is still no data for considering the toxic substance of rickettsias as a true toxin. It appears to be an exceedingly unstable endotoxin which is liberated at the moment of destruction of the rickettsias. It is interesting to note that based on the data of Pshenichnov and Mitrofanova, toxicity is observed in the capsular strains of rickettsia and is partially expressed or completely lacking in rickettsias which are devoid of a microscopically established capsule.

As a result of a critical evaluation of the above presented materials, the impression is created that all the authors were dealing with the same substance but described ic under various names depending on the properties which were detected when testing by different methods. Most likely this substance is the product from the breaking up of the unstable capsular substance of rickettsia. Further work in this direction should not only clear up the nature of the toxic substance and the antigenic composition of rickettsia, but also their role in infection and immunity with typhus. At the present time it is only possible to presumptively express the proposal that the picture of serious intoxication which characterized typhus is connected with the toxicity of rickettsia. As regards the role of the toxic substance in immunity, this problem has still not been solved. It is only known that sera of persons who had recovered from typhus, and also the sera of animals which have been immunized with rickettsias, contain antibodies which neutralize the toxic substance (Mayevskiy, 1944; Bronshteyn, 1945). Apparently no bond exists between the presence of antitoxic and anti-infectious immunity. In any case this problem requires further study. It has been established in the workds of recent years that the administration of sus-pensions of rickettsias to animals causes not only a picture of intoxication, but a series of side effects: Lowering of body temperature, leukopenia, lowering of the content of glycogen in the liver, etc. (Kligler and Olejnik, 1940; Tsal and Gunter, 1944; Cameron, 1940; Olitzki, 1941--1946). Similar symptoms may be caused in animals following the administration of cer-tain gram negative bacteria.

A feature which approximates the rickettsias with the filterable viruses is the sharply expressed cytotropism, which inhibits their culti-vation on artificial nutrient media. It is true that certain rickettsias (R. melophagi) have been cultivated, but as Weigl (1924) correctly points out, morphological similarity and development in the organism of insects serve as a very loose basis for uniting the so-called saprophytic ricket-tsias into one biological group with the pathogens. In regard to the varieties of the typhus virus (R. prowazeki, R. mooseri, R. ricketts:, R. orientalis), they, as the observations of all the investigators have demon-strated, always multiply intracellularly in the organism of man, animals and anthropods -- either in the protoplasm or in the periphery of the nuclear mass itself. We will recall that the main service of Rocha-Lima also is the proof of the strictly intracellular development of R. prowazeki, which makes it possible to distinguish the typhus causative agent from saprophy-tic rickettsias. This was not made valid by Ricketts and Wilder (1910) and Prowazek (1914). In recent years the possibility has been demonstrated of the enormous accumulation of rickettsias in the organism of various animals --

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in the membranes of the testes (Krontovskaya et al., 1943), the lungs (Durand and Sparrow, 1940; Mayevskiy, 1943), abdominal cavity (Zdrodovskiy, 1945), and in the vitelline sac of chick and duck embryos. All the attempts to demonstrate the possibility of the extracellular multiplication of rickettsia under these conditions turned out to be unsuccessful. Also unsuccessful were our attempts to achieve the growth of R. prowazeki in the dead cells of the intestine of infected lice. In 1941 Nauck and Weyer reported on the successful cultivation of R. prowazeki in explants of lice intestines, but at the same time they pointed out that the rickettsias in the cultures were devoid of pathogenicity. The latter circumstance is very strange, since in the explants of cells from mouse and chick embryos, which were obtained by the method of Zinsser, Wei and Fitzpatrick (1938), the rickettsias preserved their virulence completely, even though these cells are not the natural medium for them. From here the assumption necessarily arises that Nauck and Weyer did not obtain the growth of R. prowazeki in cultures, but of accidental, morphologically similar, organisms.

Little success has also been achieved in the attempts to cultivate rickettsia on artificial media with the addition of blood and vitamins (Klodnitskaya, 1945). There was still no success in finding substances which would speed up the growth of rickettsias. Greiff, Moragues and Pinkerton (1944), having tested a number of substances of diverse chemical nature for this purpose (riboflavin, thiamine chloride, choline chloride, biotin, physostigmine, iodoacetic acid, etc.), point out that in their tests only sodium fluoride sometimes speeded up the growth of rickettsias in vitelline sacs. However, what did its action depend on - on the depression of the protective properties of the cell or the stimulation of growth of the rickettsias themselves? It did not seem possible to resolve this. Apparently rickettsias are strongly differentiated organisms, devoid of the capability for independent metabolism. The metabolism is intimately associated with the metabolism of the living or surviving cell, and since conditions approximating living protoplasm have proved unsuccessful, attempts at cultivating rickettsias will probably not be successful. Of course such a conclusion is not absolute. Science knows many examples when following an apparent blind alley an accidental discovery, a new method or a new approach to an apparently hopeless problem produced an unexpected success.

But if rickettsias do not develop outside of a living cell, then the peculiarities of the cell metabolism should be reflected in them to some degree. In actuality the experience of cultivating rickettsias _in vivo_, often in the cells of naturally resistant animals, demonstrates their ability to endure a whole series of changes affecting both their morphological and antigenic properties. As an example some of these are "colonies--morulla" of rickettsia and odd ring-shaped, curved and nuclear forms, described in the cells of a rabbit lung (Giroud, 1945; Begg, Fulton and Ende, 1944), granules, pulverized and other forms (Giroud and Panthier, 1942; Zdrodovskiy, 1944), etc. Some authors (Zdrodovskiy and Giroud) are inclined to appraise these forms as manifestations of a particular cycle in the development of rickettsia. Our six-year observations of a series of typhus virus strains, maintained exclusively on lice (during this time several strains went through from 240 to 250 successive passages), substantiates the constancy of morphological forms and the insignificant limits of polymorphism in rickettsia multiplying in a natural medium - the cells of the intestine of body lice.

5.

We are now inclined to explain the so-called "cyclic" forms by the influence of an unfavorable medium in a resistant organism and consider them a manifestation of an involution process.

Problems of the pathogenesis of infections caused by rickettsia have been studied the least and most vaguely. While the discovery of the toxic substance shed a certain light on the origin of the symptoms of intoxication in the clinical picture of typhus, the role of the cytotropic causative agent itself, the multiplication cannot be indifferent to the affected cell, remains unclear. It is true that in the literature there are numerous attempts at detecting ricketsia in the various tissue and cells of an affected organism. This includes the works of Wolbach, Palfrey and Todd (1912), Barykin and Afanasyeva (1932), Turevich (1935) and others. But, as is known, the findings of formations which are morphologically similar with rickettsia in microscopic preparations or in histological sections does not indicate that the actual causative agent of typhus has been detected. Pathologohistological investigations reveal all the severity of the affections caused by the virus in the capillary system, however, it is hardly possible to explain all the complexity of the picture of typhus infection just by the affection of the capillaries. Recent investigations in the area of pulmonary rickettsiosis in animals demonstrated how the pulmonary tissue suffers as a result of being affected with rickettsia and how easily it becomes, due to the weakening of natural resistance, an arena of action for . banal microflora. Therefore, in the pathogenesis of typhus infection there is a great deal of interest in the problem concerning the localization of the virus (or its foci of multiplication) in the organism, and as a result of it the problem concerning the reaction of different tissues and organs to the presence of rickettsias. Up until recently attempts to clear up the role of the separate organs as foci of localization of the virus were unsuccessful or yielded results that were not too conclusive. The reason for the failures was the absence of a method which would make it possible to expose and quantitatively calculate small concentrations of virus. The common method of virus indication by means of infecting guinea pigs or infecting lice according to Weigl are not sensitive enough or are to laborious.

With the introduction of the method of epidermomembranes (Pshenichnov, 1942) and the development of a method of applying it for virus indication (Raykher, Bocharoza, Noskova, Mitrofanova), investigators found it possible to not only establish, but to quantitatively calculate the presence of even traces of virus in any material. In our laboratory Noskova (1946) used this method for studying the problem of the localization of the typhus virus in the organism of infected guinea pigs. Dissection and investigation of the organs were carried out on the 3--5th day of the fever, which set in following intraperitoneal infection with a passaged strain. Blood for the purpose of investigating for the presence of the virus was taken from the heart, after which the pig was exsanguinated by means of prolonged washing through a vascular system with physiological solution. For infecting the larvae by the method of epidermomembranes we prepared an emulsion containing 0.1 gram of organs or 0.1 cm^3 of blood from the pig in 1 cm^3 of human defibrinated blood. The average data from a number of tests showed that in the larvae which were

infected with blood, R. prowazeki were detected in 2.7%, in those infected with the liver -- in 5.03%, with the spleen -- in 13.3%, with the lungs -- in 18.8% with the brain -- in 20.4% and with the kidneys -- in 48%.

It is interesting to note that in several tests where the larvae were infected with an emulsion of organs, taken from persons who had died of typhus on the 10--22nd day of illness, the virus was detected most often of all and in large quantities in the pulmonary tissue. From here the proposal presents itself that the high concentration of virus in the pulmonary tissue indicates a particular sensitivity of the latter to the virus. Apparently also connected with this are the frequently observed inflammatory symptoms in the lungs during typhus. However, further work is required on the problem of what causes these inflammatory symptoms -- the virus itself or the secondary microflora which is taking root in the weakened tissue. A certain indication of the possibility of a specific process in the lungs is the fact that in our laboratory, following infection of larvae with sputum taken from typhus patients who had serious pneumonia, in one out of three cases it was possible to isolate a typical strain of R. prowazeki. However, it still has not been resolved how the virus reached the sputum -- from the foci of multiplication in the pulmonary tissue or from the flow of blood through the damaged walls of the capillaries. A final resolving of this problem is possible only by the joint efforts of microbiologists and pathohistologists.

A further clearing up is required of the fact of the presence of huge concentrations of the virus in the kidney tissue, especially as the damage of this organ during typhus usually does not bear a clearly expressed nature. Here it is possible to assume the presence of the emission of the virus with the urine, just as with influenza (Zilber, 1946), however it still has not been proven, in connection with the loss of viability by the rickettsia which are found in the urine. It is true that recently a work was published by Neyzhan (1945), who easily detected rickettsia in the urine of patients during epidemic typhus. However, the data in this work is so fantastic that without a sound verification it can hardly be accepted in trust. We recall that during murine typhus the possibility of the elimination of the virus with the urine of sick rats was demonstrated experimentally in the works by Morgandier and Pirot (1933) and Nicolle, Giroud and Sparrow (1934).

While much in the pathogenesis of typhus remains unclear for us, the main epidemiological problems have been almost completely resolved. In 1909 Nicolle, having established the body louse as the carrier, spoke out for the transmission of the infection through bites, and only considerably later came to the conclusion that "scratching the skin with the nails which were contaminated with the excrement of lice was just as capable of transmitting the disease as the bites." Soon however the majority of investigators (Atkin, 1922; Arkrayt and Bako, 1923;Sikora, Mozing and Radlo, 1938; Rshenichnov, 1940; Gromashevskiy and others) refuted the opinion that the bite is the moment of the transmission of the infectious onset, and pointed out that the transmisstion of typhus is possible only through the rubbing in of the excrement

of infected lice. Thus, for example, Mozing and Radlo (1938) write, "Infection with epidemic typhus does not take place through the bite of an infected louse, since the mouth organs of the latter do not contain the virus. Due to scratching, man makes it possible for the excrement of lice to penetrate the epidermis, which in like manner to the penetration of infected excrement of lice into the conjunctive, may cause infection." This proposal was fully supported in the work at our laboratory. By feeding ten thousand massively infected lice on the epidermomembrane, we showed that the sterile blood which was located under the membrane never became infected. The use of even the most sensitive indicator -- the larvae of body lice, never disclosed traces of the virus in it. However, by placing the virulent excrement of lice on the epidermomembrane and subsequently feeding healthy insects on it, this always caused the infection of the blood under the membrane, apparently due to the entry of the virus with infected mouth organs or into the openings at the site of bites (P..enichnov, Raykher, Kazakova). The prolonged preservation of the virus in excrement and dead lice, which has been demonstrated by a number of authors (Arkrayt and Bako, 1923; Starzyk, 1938; Feigin, 1938; Pshenichnov, Raykher, 1939; Pshenich-nov, 1940; and others), in connection with the easy penetrability of the mucosa (Blanc and Baltazard, 1944; Sparrow and Lumbroso, 1929; and others) is a compelling force to accept the probability that along with the rubbing of excrement into the skin, the infection may also set in if the excrement reaches the mucosa of the eye and the upper respiratory tract. The resistance to desiccation on the part of the virus which is found in the excrement makes it possible, in an analogy with aerogenic infection during experimental pulmonary rickettsiosis in animals (Krontovskaya et al., 1940, Mooser and Loffler, 1942), to suspect that in individual cases aerogenic in-fection is also possible following the dispersion of the excrement of lice. There are indications of such a possibility in the literature (Grenoyl,1945, Kloze, 1942), however, they do not bear the nature of experimental or epi-demiological proof. The data which are obtained during laboratory infections, where infection is carried out with massive doses of the virus, cannot be transferred into epidemiological practice. As regards the problem concerning the length of stay of the virus in the blood of man, it has been estab-lished now that infection by lice is possible on a patient, beginning with the last days of incubation and ending with the first days of convalescence (Gromashevskiy et al. 1939; Raykher, 1939; Mitrofanova, 1945). During the incubation period only a very limited number of feeding lice are contaminate due to the small concentration and the inconstancy of the virus in the blood. The maximum of the susceptibility of infection of the lice is reached in the first week of illness and is progressively lowered by the period of convalescence. As a rule a drop in temperature is accompanied by the com-plete riddance of causative agents from the blood and only in detached case it can be detected on the 2nd (Gromashevskiy) or 2--5th (Raykher) day of convalescence. The works which speak of the presence of the causative agent in the blood after several months and even years following the disease were the results of an insufficiently critical approach to experiments on guinea pigs.

In the course of recent years the investigations at our laboratory have

cleared up the epidemiologically important dependency between the conditions for the contamination of lice on the patient and the period of the development of the virus on them, that is, the period of the appearance of the capability to transmit infection.

It was possible in a number of case to show that the development of the virus in lice depends on two main conditions: The infectious dose obtained at the moment of infection, and on the temperature regimen of the medium of nourishment of the insects. Depending on these conditions the time for the appearance of the virus in infected lice may fluctuate from 3 up to 18 days from the moment of infection. From here it follows that the periods of development of the virus in the lice which were indicated by authors earlier (Nicholle - 8 days, Rocha-Lima - 5-6 days, Shukhat and Revich - $3\frac{1}{2}$ days) were determined only by the conditions of the experiment. The infectious dose and the temperature of the medium also determine the period of life of the infected lice, that is, the period during which they are capable of taking an active part in the transmission of the infection. Under the conditions of the experiment, depending on the dose taken for infection of the lice and on the temperature at which they are maintained, the lice die within the 5th and 31st day. The reason for the death of the lice at the height of the development of the rickettsia apparently is not only the damage to the wall of the intestine, which is accompanied by the phenomenon of hemolytic imbibition ("red lice"), but also intoxication.

Long ago the problem of the so-called "interepidemic period" interested investigators. The beginning of this problem is laid out in the works by Mooser in Mexico, in which on the basis of a number of epidemiological and experimental observations it is maintained that the preservation of the virus between epidemics is possible in rats, and the transmission of the infection may be carried out in the following chain: Rat - flea - man - louse - man. At one time this point of view was adjoined to by Nicolle, who, following a meeting with Mooser, wrote that the discovery by Mooser "makes it necessary to suspect the rat, and certainly other domestic rodents and their parasites, of playing a specific role in supporting the virus, and possibly in the emergence of epidemics." However, subsequent works by a number of authors (Zinsser, Castaneda, Blanc and Baltazard, and others) showed that the point of view expressed by Mooser was the result of a misunderstanding caused by the simultaneous absence of epidemic and murine typhus in Mexico. Also no confirmation was found for the works of numerous authors who attempted to find a natural reservoir for the virus among different animals (cats, sheep, birds, etc.). It can be considered as solidly established that the natural source of infection during epidemic typhus is a sick person, and under natural conditions no animals can be the custodians of the virus. The great significance of this proposal for epidemiological practice is not lacking in proof.

As regards the so-called "degree of virus carrying" in man, the hypothesis put forth by Sh. Nicolle on the basis of the presence of a symptomless infection in guinea pigs, which was noncritically picked up by a number of investigators, should be accepted as absolutely unsubstantiated. This is spoken for by the epidemiological observations of Gromashevskiy and associates and the recent experimental data from our laboratory (Pshenichnov

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and Pshenichnova, 1945). The use of body lice larvae, even under conditions of the membrane-passage method (Mitrofanova, 1945), which discerns a thousand times less dose of virus than the dose which is necessary for the infection of guinea pigs, could not expose the presence of the virus in an immune organism which has been subjected to a secondary mass infection.

At the present time no confirmation has been given to the point of view of Zinsser, who views Brill's disease as a regeneration of typhus which had been endured in the distant past. Even Blanc and Baltazard, who defended the presence of symptomless infection in man, caused by the murine virus, categorically rejected the point of view of Zinsser on Brill's disease as unfounded. The typhus virus is not transmitted by heredity from infected body lice to their progeny. The investigations of our laboratory (Pshenichnov, Raykher, Subbotina) showed the R. prowazeki, the Volhynia fever rickettsia and saprophytic rickettsioses are not transmitted in lice by the transembryonal route.

All of this compels a specific, practically important, conclusion concerning the fact that a so-called interepidemic period does not exist. The typhus virus is stored between outbreaks only in those natural cases of typhus which (particularly often in children) remain unrecognized and unhospitalized.

In the coming years the Soviet public health service will undertake the mission of the complete liquidation of typhus in our territory. The early, simple diagnosis of this infection is one of the paths leading to the achievement of this goal. In actuality, only the early diagnosis guarantees the early hospitalization, and the latter prevents the dissemination of the typhus virus to the public and ensures the timely treatment of the patient. Since the time of the first World War all diagnosis of this infection essentially amounted to only the Weil-Felix reaction. Numerous modifications of this reaction, proposed both earlier and in recent years (Knyazhanskiy, 1936; Rubenshteyn, 1943; Shkorbatov, 1943; Leon, 1945; Castaneda and Silva, 1938 and many others), did not introduce anything principally new into this reaction and only simplified setting it up and brought it closer to the bed of the patient. Practice has shown that the results of the Weil-Felix reaction and its modifications should always be accepted with great care (Ragoza, 1946). At the same time in some cases (5.7%) this reaction yielded negative results when checked on a great deal of material (Berk, 1946). For increasing the diagnostic value it was proposed that a whole series of Proteus strains be introduced into this reaction (Berk, 1946) as well as many other laborious innovations.

It seems to us that now further experimentation on the Weil-Felix reaction in searches for new modifications and "improvements" is hardly promising, since it should not be forgotten that this reaction is not carried out with the causative agent of typhus. The Weil-Felix reaction occupied and will continue to occupy its proper place in the laboratory, but the further improvement on the diagnosis of typhus should be built on new principles and go along new paths.

10.

For replacing the Weil-Felix reaction in the search for antibodies in the sera of typhus patients it was proposed to use the specific antigen -- R. prowazeki. Smorodintsev and Drobyshevskaya, 1943; Bengston, 1946, and others, having used this antigen in the complement fixation reaction, noted the great specificity of this diagnostic method.

Much earlier R. prowazeki were used in the agglutination reaction. Having proposed the micro-agglutination reaction, Weigl (1924) proved that this reaction was specific and was manifested in the patients 1--2 days earlier than the Weil-Felix reaction. However, the complexity of preparing the antigen, its weak stability, and mainly the unavoidable subjectivity in evaluating the results of this reaction did not ensure its wide application. Pshenichnov and Raykher (1944) altered this reaction and proposed their modification in the form of the so-called light micro-agglutination reaction. The antigen for this reaction is fully accessible, since it is prepared from typhus lice which had been infected by the method of epidermomembranes. The period of suitability for the antigen is no less than 4--5 months. In evaluating the results of this reaction the subjectivity of the investigator is reduced to a minimum. The technological process of the reaction amounts briefly to the following. Various dilutions of serum are prepared and a drop of each is placed on a slide. A drop of antigen is added to the serum, then the slides are enclosed in a moist chamber and set in an incubator for one hour. After this smears are made form all the drops and stained according to Romanovskiy-Giemsa. Under immersion it is possible to observe either a clear agglutination or rickettsia uniformly scattered in the preparation. A check of this reaction (Faydysh, 1946) showed that it appears in the patients 1--2 days earlier, gives higher titers than the Weil-Felix reaction, and in contrast to it in small titers is seldom positive in healthy persons. It seems to us that this reaction will find application in the serological differentiation of various types of rickettsia. It is interesting to note that Giroud (1944), almost simultaneously with us, proposed his modification of the Weigl reaction, which in many respects is analogous to ours, but is set up with an antigen which contains the rickettsia from the pulmonary tissue of animals. The antigen, prepared from rickettsia, for setting up the macroscopic agglutination reaction, was proposed by Mayevskiy and is used in his laboratory.

However, all the diagnostic reactions, which are set up with an orientation for searches for antibodies, are retrospective. In this is their main and non-adjustable shortcoming -- they do not guarantee that early diagnosis which would make it possible for the doctor to make a diagnosis of typhus in the first days of illness. For diagnostic purposes Mayevskiy and Ratner, Giroud and others attempted to use allergic tests with the lysates of R. prowazeki. With this same aim Bishafer (1943) recommends the neutralization reaction. But the experience of working with other infections teaches us that it is doubtful that these reactions, due to their instability, will bring us to a solution of the problem, especially as the typhus antigen itself with its ephermeral toxic substance is far from promising for these purposes.

It is necessary to recognize as absolutely correct and logical a new trend in the laboratory diagnosis of rickettsioses and viruses with an

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orientation to the antigen--causative agents, which appear and accumulate in the human organism during the development of infection. The works of Ioffe, Smorodintsev, and Dropyshevskaya showed for the first time the feasibility of utilizing the complement fixation reaction in the cold for detecting the typhus antigen in sera taken from patients in the first days of illness. Leon and Carmen (1945) supported the data of Soviet investigators and showed th-t a positive reaction is detected in all patients prior to the 10th day oi illness.

In principle our laboratory agrees with the feasibility of detecting the antigen in the sera, but at the same time we established that the stated reaction is very complex, prolonged, and does not always give positive results even in the first days of illness. This is explained most rapidly of all by the fact that with some of the patients in the small amount of serum which was taken for investigation the amount of antigen was so insignificant that is is not detected even by such a highly sensitive method as the complement fixation reaction in the cold. In introducing the problem concerning the early diagnosis of typhus, it was necessary to assume that in the blood, in this naturally possible source for searching for the virus in the patient, the R. prowazeki are distributed unevenly, since the composition of the blood is heterogenous and consists of plasma and cellular elements. Nicolle already noted that the sera cf typhus patients often did not contain the virus, but on the other hand Kosmodamianskiy (1938) confirmed that in leukocytes its concentration is comparatively high. At the present time these observations are ignored and for seeking the antigen in the blood of a typhus patient only the sera are used. It should not be forgotten,however, that R. prowazeki possess cytotropism, and the erythrocytes of man are capable of adsorbing bacterial toxins (Zbarskiy, Zilber, Melnikov), bacterial haptens (Kravcheno) and the influenza virus (Herst). Thus, before taking serum as the object of investigation it was necessary to resolve the problem of where the typhus virus is localized in the blood, For these purposes our laboratory (Noskova) used the method of epidermomembranes, and as the biological agent for typhus -- lice larvae. The blood from sick persons or guinea pigs was taken in the first days of illness. After coagulation or defibrination the serum of a healthy man was added to the ground clot or the washed formed elements of the blood being investigated, and vice versa, the formed elements from a healthy man were added to the typhus serum. Both mixtures were introduced under the membranes and a large series of lice larvae were fed on them one time. Then the infected insects were investigated for the presence of R. prowazeki and based on the percentage of positive findings conclusions were made concerning the distribution of the virus in the blood of the sick organism. It turned out that the lice were not infected with the sera from the typhus pigs and the infection rate for the formed elements of the blood fluctuated from 1.6 up to 5.5%. Data close to this was also obtained with the blood from typhus patients. Following the intravenous administration of a massive dose of rickettsia to a rabbit and an investigation of the blood after 30 minutes, the formed elements of this blood, even after a threefold washing with physiological solution, caused the infection of 62.3% of the lice, and the serum -- only 46%. These data led to the conclusion that typhus sera are extremely poor in virus, that the main mass is intimately bound with the formed elements of the blood. Then it was completely natural that the problem should arise concerning the development of

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new diagnostic methods with an orientation to antigen from the formed elements of the blood. Stemming from this, Petrova (1946), having obtained defibrinated blood from patients, made an attempt in the very first days of typhus to set up, using the ordinary method, the complement fixation reaction both with sera and with washed erythrocytes. It turned out that the sera did not produce thephenomenon of complement fixation. On the other hand in a number of cases the erythrocytes partially fixed complement, which was exposed during its subsequent titration. The new principle for the early diagnosis of typhus found further support in these tests.

"The presence of immunity following a case of typhus serves as a foundation for the carrying out of active immunization," wrote Sh. Nicolle (1935). Though this provision was known to investigators for a long time, the development of the problem of vaccination against typhus moved very slowly. This was explained by the fact that many microbes were used as the culprits of typhus infection, and the true culprit -- R. prowazeki -- was exposed very slowly. However, even the discovery of the causative agent had little effect on the position, since the rickettsia turned out to be biotropic and were cultivated with great difficulty. Numerous vaccines, such as the blood of patients, emulsions from typhus pigs and others, which were proposed prior to the Second World War, turned out to be ineffective due to the insignificant amount of antigen included in them. However only one vaccine from typhus lice, proposed in 1917 by Rocha-Lima and improved by Weigl by means of applying the method of artifical infection of insects (enema method), received approval, however, this was not a vaccine for mass application. The Second World War, and with it the new threat of typhus, brought special urgency to the problem of developing an effective typhus vaccine which could be produced on a mass scale. During the war the development of vaccines from killed virus went along the following main trenads. In 1936--1939 Castaneda, and independently from him Durand and Sparrow in 1940, found that following the intranasal administration of rickettsia in white mice a specific pneumonia developed which was accompanied by the profuse multiplication of the causative agent in the pulmonary tissue. This discovery made it possible for Durand and Giroud (1940) to create the so-called "pulmonary" typhus vaccine.

In the USSR the mass production of "pulmonary" vaccine was mastered by Krontovskaya and Mayevskiy. The latter, in contrast to Durand--Giroud, used the historical virus from the intestines of lice for adaptation to mice. Krontovskaya adapted the virus from membrane of the testicle of pigs, having caused in them a scrotal phenomenon by the intraperitoneal administration of large quantities of typhus lice. During the period of the war the "pulmonary" vaccine was also used in France, Switzerland, Rumania, Germany, Tunisia and other countries. Subsequently it turned out that pulmonary rickettsiosis may also be obtained in other animals, and therefore Durand and Sparrow (1941), Castaneda (1939) and Zdrodovskiy (1943) suggested that vaccine be prepared from the lungs of rabbits, Combiescu (1942) -- from the lungs of dogs, Giroud (1945), Horrenberner (1946) -- from the lungs of sheep and goats. Besides the "pulmonary" vaccine from "washed" rickettsia, Muromtsev, (1943) proposed the so-called "tissue" from finely ground lungs. Giroud confirmed the advantagousness of preparing "tissue" vaccine, having demonstrated that the tissues of the lungs, even after the

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removal of the rickettsia, preserved high antigenic properties. Castaneda (1942) suggested a bivalent vaccine, containing the historical and the murine virus. On the basis of his investigations he deems that this vaccine imparts a more permanent immunity.

During the war mainly new changes were introduced in the technology of manufacturing vaccine from typhus lice. The method of epidermomembranes (Pshenichnova) made it possible to produce infected insects in huge quantities, and from this it became possible in the USSR to produce millions of man-doses of a new modification of the vaccine -- the vaccine of Pshenichnova-Raykher.

The vaccine of Weigl was produced mainly in Germany and Poland. As is pointed out by Weigl (1947), recently around 5--6 million men have been inoculated. In spite of the fact that due to the conditions of the military situation the vaccine was used in a lowered dosage (25--30 lice intestines per one man in place of 100 intestines), its effectiveness was very high and "exceeded all expectations." Ignazio (1945) also reported of the high effectiveness of Weigl's vaccine, which produced a "remarkable" effect against a background of a general lethality of 21% among noninoculated persons.

A new phase in the creation of vaccines from cultures of rickettsia emerged in connection with the works by Gudpaschur. In 1940 Cox and Bell, proceeding, as they point out, from the works by Barykin (1935), suggested the cultivation of rickettsia in the vitelline sac of a chick embryo and used these cultures for the preparation of vaccine. The later works by American authors showed that the concentration of antigen in the vaccine prepared according to the method of Cox is insufficient. In connection with this, Craigie developed a new method which is accepted now in the large industries of the USA and Canada. The chief peculiarity of this method is the extraction of an emulsion of vitelline sacs. During this the specific thermolabile antigen is extracted from the rickettsia. The ether extract, from which the ether is removed, is also a vaccine. Titration on eggs showed that the concentration of rickettsia in the preparation, according to the data of Craigie equaled 10^{-13}, and according to the data of other authors - 10^{-8}. In the USSR the method of Craigie was used successfully by Mayevskiy for the preparation of "pulmonary" vaccine. In spite of the fact that the egg vaccine of Craigie contained more antigen, is subject to detoxication, and purified from slag, based on recent literary data it does not have any significant advantages over other vaccines. On the other hand, following the carrying out of extensive vaccination in the Belzensi camp, Davis (1945) and others gave the preference to the vaccine of Cox. During the period of the war the "egg" vaccines were used widely: In Western Europe alone for 1945 more than 2 million inoculations were given. These same vaccines were introduced into the British and American armies. However an unexpected complication in the use of egg vaccines turned out to be the severe reaction in persons who possess an increased sensitivity to egg protein. In the literature there are even descriptions of lethal outcomes as a result of severe anaphylactic shock (Rifkin, 1946; Hampton, 1947). There is theoretical, if not practical, interest in the vaccine of Zinsser-Fitzpatrick, which is prepared from rickettsia grown on agar-tissue media.

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The widely conducted vaccination against typhus created the necessity for checking the effectiveness of various vaccines. On the basis of an extensive analysis of materials on this problem, the conclusion can be made that all the modern vaccines are probably equivalent based on their effectiveness. Topping (1945), Levkovich and many others arrive at this same conclusion. The vaccines have caused a lowering of incidence by 3--7 times. A doubtless result of the vaccination is the lowering of the severity of the course of typhus in inoculated persons. In these persons the infection proceeds benignly, mildly. In many hundreds of thousands of persons inoculated with "pulmonary" vaccines only several cases of death from typhus were recorded (Durand, Fayershteyn, Los, Shvartsman), while the lethality in noninoculated persons fluctuated from 5 up to 20%. In Egypt following the mass vaccination by the method of Cox-Craigie, there was only one case of death per 60 incidences among inoculated persons while the lethality among noninoculated Egyptians was 18%. In the inoculated group there were no serious illnesses, while 2/3 of the noninoculated had a serious form of the disease. Fox (1946) notes that following vaccination with the Craigie-Cox vaccine not one case of death from typhus in the armies of the allies is known to him. Felix, Tokarevich, Koryakina and others also did not notice one case of death in persons inoculated with vaccines from typhus lice.

In generalizing the results of the application of typhus vaccines, it is necessary to recognize that in the Second World War they played an important role in combatting typhus; They were mainly responsible for resolving the problem concerning the feasibility of using killed rickettsia for vaccination, but from here there cannot be an absolute conclusion that these vaccines may replace the main antiepidemic measures such as early hospitalization and lice extermination in foci.

In spite of the successes achieved in the development of vaccines, it is necessary to recognize that they are still far from ideal. There are still no reliable methods for determining the quality of typhus vaccines. The method of the quantitative calculation of the inoculation material, the determination of the amount of rickettsia in smears, optical standardization, cutaneous test, and the determination of the effectiveness of the vaccine on pigs have not given satisfactory results in laboratory practice. The main difficulty in resolving the quality of a vaccine is found in the fact that rickettsia are very fragile and are easily lysed, and due to this are not subject to computation, in spite of the fact that such material preserves the antigenic properties (Topping, Shear, Giroud, Raykher). Attention is merited to the further development of new methods for studying the effectiveness of vaccines, such as: The method of titration of agglutinins (Mosing, 1946), determination of antitoxic properties on mice (Henderson, 1943, Mayevskiy, 1945; Fitzpatrick, 1945), and the utilization for this purpose of the complement fixation reaction (Vikoff, 1942).

The problem concerning the epidemiological significance of typhus in inoculated persons   requires conclusive resolution. Contradictory information exists on this problem. Thus according to the data of Weigl and Smorodintsev, lice are not capable of being infected on inoculated persons

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who are going through a light form of typhus, and on the contrary Mitrofanova and Heldner argue the possibility of infection of individual specimens. Either the restriction of the scope of vaccinations or the granting of civil liscence to all vaccines without consideration of the sanitary condition of the focus are dependent on the solving of this problem. Without a doubt far from all has been taken from killed typhus vaccines, and therefore, the improvements of these vaccines which are being contemplated at the present time merit attention and further work. Examples of these are preparations of associated vaccines (Laigret, Los), dry vaccines from typhus lice (Osadinova), and sorbed vaccine (Pshenichnov, Topping, Bengston).

The live vaccine of Blanc, which contained the murine virus, was suggested for use against typhus. In 1944 Blanc and Baltazard wrote that "between the murine and human viruses there is much in common, their drawing together. Between themselves all these viruses create an absolute immunity without any differences in antigen capability." However, recent works (Hudson, 1940; Topping and Bengston, 1943; Castaneda, 1945; Hamilton, 1945; Craigie et al., 1946) have established quite firmly differences in the antigenic structure of this and that virus, and by this have shown that it is difficult to expect to obtain a full value immunity to epidemic typhus as a result of vaccination with murine strains. Based on the data of Lemaire (1943) and others, the Blanc vaccine caused a lowering of incidence among those inoculated by no more than 5--8 times, and according to Durand (1943) it turned out to be less effective than killed vaccine. At the same time this vaccine, just as during the well-known catastrophe in Chili, continues to cause a severe experimental typhus in those inoculated (Lopez, Soviano, 1943 and others). Therefore, the final resolving of the problem of vaccination during typhus will be the obtaining of an avirulent strain of the virus fixe type, but from an epidemic virus. This path is promising but prolonged and thorny. Already in 1924 Weigl wrote that by cultivating the virus just on lice it is possible to obtain a virus which is apathogenic for guinea pigs. Following the four year cultivation of one strain on lice our laboratory (Pshenichnov, Raykher) also obtained an attenuated modification of the virus. This virus was used with success for the vaccination of guinea pigs and caused an intense immunity to subsequent infection with a strain which was freshly isolated from a typhus patient. Following the accidental infection of one nonvaccinated laboratory worker such a light disease set in that the temperature never reached 39°, held out 7 days, and the patient literally endured typhus on his feet. Of course, this is far from that typhus apathogenic variant which Sh. Nicolle always makes note of, but the path taken by us, that is, the cultivation of rickettsia outside the organism of warm-blooded animals, with the influence of unfavorable temperatures on it, seems promising to us. It is interesting to recall that in 1941 Cox, after 100 passages on vitelline sacs, transformed one strain of spotted fever into a virus which was attenuated for pigs, and made the proposal concerning the possibility of using it as a vaccine. A great sensation was the report by Clavero and Gallardo (1943), who after 16 passages obtained a strain which lost its pathogenicity for pigs; in 1944 they used a live vaccine from this virus to vaccinate 2,217 persons in Spain. In all of those inoculated a

light form of typhus was observed in only one. However, no further support for these works was obtained. We consider that the world is waiting for a full value typhus vaccine, but which of the investigators will be first to achieve this and by which method remains to be seen.

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